Modeling AFM Induced Mechanical Deformation of Living Cells

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ABSTRACT

Finite element modeling has been applied to study deformation of living cells in Atomic Force Microscopy (AFM) and particularly Recognition Force Microscopy (RFM). The abstract mechanical problem of interest is the response to RFM point loads of an incompressible medium enclosed in a fluid membrane. Cells are soft systems, susceptible to large deformations in the course of an RFM measurement. Often the local properties such as receptor anchoring forces, the reason for the measurement, are obscured by the response of the cell as a whole. Modeling can deconvolute these effects. This facilitates experimental efforts to have reproducible measurements of mechanical and chemical properties at specific kinds of receptor sites on the membrane of a living cell. In this article we briefly review the RFM technique for cells and the problems it poses, and then report on recent progress in modeling the deformation of cells by a point load.

Keywords: finite element modeling, biological cell, cell membrane, living cell, AFM

1 AFM AND LIVING CELLS

The current bioscience revolution has relied on the introduction of new technology to characterize biological systems. One of the current challenges is the development of technology for characterization at the cellular and sub-cellular level. This is needed for the direct study of how genome information is expressed at the microscopic level, for example. It could also prove useful in presymptomatic detection of infection. Scanning Probe Microscopy has been identified as a promising means to characterize biological systems at scales of nanometers to microns.[1] Atomic Force Microscopy (AFM) and its derivatives such as Recognition Force Microscopy (RFM) are well suited to the characterization of biological systems [1]. AFM uses the deflection of tiny cantilevers in contact with the specimen to provide information about the topography and elastic properties of cells; RFM goes a step further, using molecules attached to an AFM cantilever tip to study the binding at various sites on the specimen. In particular, recent studies have measured the deflection of the AFM cantilever during approach and retraction in order to find the unbinding forces for various ligand-receptor pairs, such as biotin-avidin [2], paired DNA bases [3], antibody-antigen complexes [4] and cell recognition proteins [5]. This paved the way for experiments using a single receptor molecule bound to the AFM tip to map the location of ligands bound to solid surfaces [6]. It is only now becoming possible to use the techniques on living cells, and this has been the aim of our group at Lawrence Livermore National Laboratory [7].

One challenge with using RFM on living cells, as opposed to molecules fixed to a surface, is the fact that the cell is not rigid. As force is applied to a receptor site, it is not just the receptor site that is affected. The whole cell deforms under the applied force. The measured force-distance response is a convolution of the local, intrinsic response of the receptor and the gross elastic response of the cell. We have developed new computational tools, and to some extent new models, to study the deformation of the cell and separate the two effects.

The modeling is based on a continuum level analysis of the elastic deformation [8], implemented in a finite element model [9]. The model is unconventional because membrane bending makes a major contribution to the strain energy. The appearance of the membrane curvature in the energy necessitates the use of finite elements with shape functions whose first derivatives are continuous everywhere; i.e. conforming C^1 finite elements. The computational model is discussed further below.

2 STRUCTURE OF THE CELL

The bovine sperm cell has been selected as the model system for our experiments to develop the AFM and RFM techniques on living cells. An AFM topographic scan of the cell is shown in Fig. 1. The figure is a perspective view of a single sperm cell generated from an AFM scan in which height is measured in tapping mode as a function of x-y position [7].

The sperm cell was chosen for these experiments because of its well defined shape, because its structure and composition have been well characterized and because it can be triggered to undergo a transformation in which receptor sites become active on the exterior

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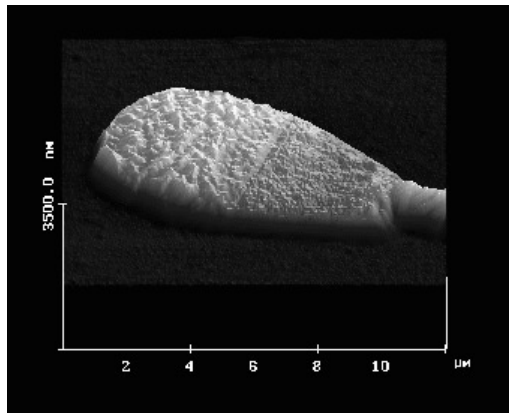[1]Length units: 1 micron = 10^{-6} m.

Figure 1: Topography of a living bovine spermatazoum immersed in buffered water at room temperature from a force-volume scan by atomic force microscopy (AFM). [7] Note the relatively well-defined shape. The head may be seen to have different regions: the acrosomal region in the front, the equatorial segment and the post-acrosomal region near the tail.

membrane of the cell. The sperm cell has a relatively simple anatomy [10]. It is well suited for its purpose, to transport DNA for reproduction. Most of the head is packed with chromatin (proteinated DNA). Roughly half of the volume of the head is occupied with chromatin; the other half is water. The chromatin helps define the cell shape, which is long and flat, roughly 10 microns long by 4 microns wide by 0.5 microns thick. The cell membrane also contributes to the shape. The membrane consists of three to five phospholipid bilayers, depending on the site on the cell. The front of the head, the acrosomal region, consists of three bilayers. The section of the head near the tail, the post-acrosomal region, consists of two bilayers. Differences in the membranes in different regions of the head are clearly evident in Fig. 1. Apart from the nucleus, the only organelles in the head are mitochondria located near where the tail attaches.

Our goal is to use AFM and RFM to investigate the properties of specific receptor sites on the cell membrane. Currently, we can observe binding events on a living cell in which the molecule attached to the AFM tip binds to a receptor site, and then ultimately unbinds as the AFM tip is retracted. These investigations are continuing, but it is beyond the scope of this Article to describe them in any detail. Nevertheless, it should be clear that if the cell is deformed as a whole when a particular site is probed, then the contribution of the gross deformation must be subtracted in order to find the intrinsic properties of the site. We have constructed a mechanical model of the cell, as described below, to allow us to calculate the gross cell deformation induced by the AFM tip. Several kinds of AFM measurements will be needed to parameterize and validate the model.
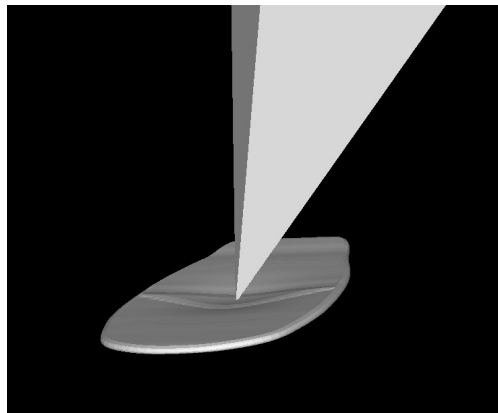

Figure 2: The model geometry of the indented cell and the AFM tip. The length of the cell body and the width of the AFM cantilever are both about 10 microns. The radius of curvature of the membrane about the AFM tip has been computed to be about 1 micron. [7]

One set of experiments that has been completed is a measurement of the force vs. distance curves during AFM indentation of the cell [7]. A model of this process is shown in Fig. 2. These experiments were conducted using a liquid cell AFM in which the sperm cell was immersed in a buffered aqueous solution during the measurements. During indentation and retraction, the displacement of the piezotube that actuates the AFM and the resulting deflection of the AFM cantilever were measured. The force was determined from the cantilever deflection using the known spring constant for the cantilever. The result was a measure of the elastic properties of the cell as a function of the depth of indentation. In the shallow indentation regime the effective stiffness was measured to be 0.03 N/m [7]. This value increased for indentation beyond half of the cell's thickness.

3   MODELING MECHANICAL DEFORMATION OF CELLS

The load caused by retraction of the cantilever in RFM or the indentation by an AFM tip causes deformations of the cell that extend over distances that may approach the size of the cell, and are large compared to other length scales in the system such as the separation between neighboring lipid molecules in the membrane or the membrane thickness. In the case of a 0.3 micron indentation, the radius of curvature of the deformed membrane has been computed to be about 1 micron [7] (see Fig. 2). On this relatively large length scale, continuum mechanics is appropriate to model the deformation. Were we interested in the indentation of a solid surface, conventional techniques such as Hertzian theory [11] could be employed to extract the elastic constants of the material. This has been employed in the

case of cells as well [12], but it has proven problematic because of the large deformations and the finite size of the cell [13]. Hertzian theory, for example, assumes that the system undergoing indentation is a half-space, a semi-infinite system bounded by a flat surface (before indentation). This is not a good approximation for cells. As noted above, the radius of curvature of the membrane about the AFM tip is a substantial fraction of the cell's horizontal and vertical dimensions [7].

As discussed above, the response of the cell during indentation shows an initial soft resistance followed by a more rigid resistance, and this has been interpreted as two distinct regimes of deformation. In the first regime, the resistance is viewed as due to bending of the membrane to accommodate the indentation and the incompressibility of the interior; in the second regime, the resistance has been proposed to arise from the compaction of the chromatin during the deep indentation [7]. For RFM applications, the shallow indentation regime is relevant. With this guidance from experiment, we have developed a continuum model for the cell deformation [7]. The interior is modeled as an incompressible fluid and the tip as a point force. The membrane is assumed to be under-inflated so that the tension is zero. Even so, it costs energy to deform the membrane because of its bending rigidity. The strain energy of the membrane is taken to be of the Canham-Helfrich form [14],

$$W = \frac{\kappa}{2} \int dA \left(H - H_0\right)^2 \qquad (1)$$

where H is the mean curvature of the membrane, $dA = \sqrt{g}d^2x$ is the area element with g the determinant of the induced metric, and κ and H_0 are material constants, the bending rigidity and the intrinsic curvature, respectively.

An interesting issue arose regarding the strain energy. Fluctuations of under-inflated membranes have been studied extensively and modeled using the Canham-Helfrich energy which gives the energy of the membrane as a function only of its current shape [8]. Another approach has been to treat the membrane using Kirchhoff-Love plate theory [15]–[17], as would be used to describe the deformation of a steel plate. It describes the deformation in comparison to a reference configuration. It has recently been shown that the two formulations are equivalent to the extent that they predict the same deformations provided the membrane remains in equilibrium with respect to tangential deformations [18]. The formalism without a reference surface is simpler, so it is the one we have implemented.

The curvature that enters the Canham-Helfrich energy (1) is given by a second-order partial derivative of the displacement, so the equation of mechanical equilibrium is a fourth-order partial differential equation. It has been studied by several groups in the axisymmetric case, where the equation reduces to a fourth-
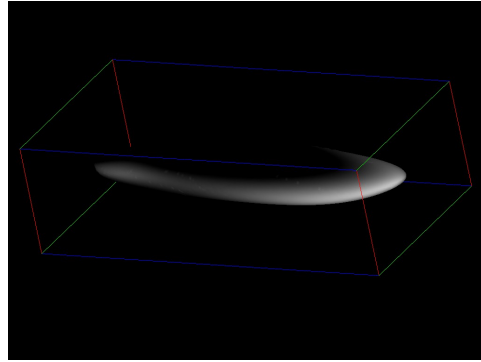

Figure 3: A representation of the membrane energy landscape: the contour surface of fixed reduced volume, in Legendre space (see text). The contour is shaded according to the bending energy, with white for the minimum of energy (the equilibrium shape).

order, non-linear ordinary differential equation (ODE) [17] or equivalently a set of coupled, non-linear first-order ODE's [19]. The point indenter produces a curvature singularity that requires special treatment or regularization. Otherwise, the deformation is smooth and can be computed using a C^1 finite element technique.

A new formulation of finite elements based on subdivision surfaces has been developed by Ortiz, Schröder and coworkers recently for surfaces in three dimensions [20]. These shape functions are just smooth enough to ensure that the bending energy (1) is well defined and converges rapidly. We have used the one-dimensional analog of the subdivision elements, cubic splines, to calculate the deformation of axisymmetric vesicles in equilibrium and in indentation. A difficulty in the modeling is the existence of local minima in the strain energy as a function of membrane shape. Local minima give rise to interesting physical effects such as the non-linear flicker seen in some vesicle systems. In order to investigate the energy landscape, we have projected the equilibrium shapes onto the space of symmetry-allowed Legendre polynomials. Fig. 3 shows the isosurface of constant reduced volume, $V_{red} = 10.6$ V/A$^{3/2}$ = 0.78, colored according to the energy. It shows that apart from multiple branches, the energy landscape is not particularly complicated, and certainly not pathological.

4   BEYOND FINITE ELEMENTS

The interaction of the binding molecule with the receptor site is currently modeled as a point force. It would be desirable to implement a more detailed model of the binding, perhaps even modeling the receptor site at the atomistic level. Concurrent multiscale modeling may provide a means to do this and at the same time model the larger-scale mechanics of the membrane and cell deformation [21], [22]. The principle of multiscale

modeling is to use different models in order to describe the physics of the system at different length and time scales. It has been shown that atomistic models can be coupled directly into continuum models such that they run concurrently [21]. This provides a more detailed description of the system where it is needed, while retaining the efficiency needed to model the entire system.

The RFM system would be an extremely interesting application of concurrent multiscale modeling techniques. The binding of an antibody or ligand to a receptor site could be modeled atomistically, along with the portion of the membrane and solvation layers immediately around the receptor site. This would then be coupled into the continuum description of the rest of the cell that has been described above. This would allow a very precise study of binding effects that would not need to neglect the contribution of the cell deformation as a whole. To date, only conventional C^0 finite elements have been coupled to atomistics, so a concurrent methodology based on C^1 elements appropriate for membranes would require some development.

The use of RFM on biological cells poses some interesting challenges for modeling. The unusual form of the energy has required an unconventional computational approach. So far the model has been used to study vesicles as test cases, but it will soon be possible to apply it to actual RFM data. This new window into the mechanical and chemical properties of receptor sites promises new discoveries that will deepen our understanding of cell function.

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